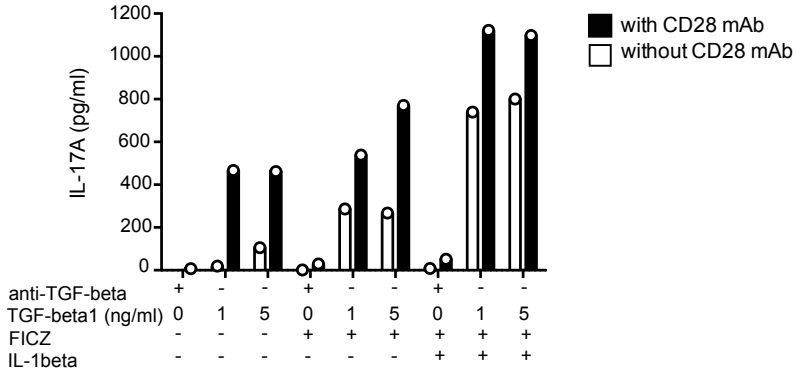
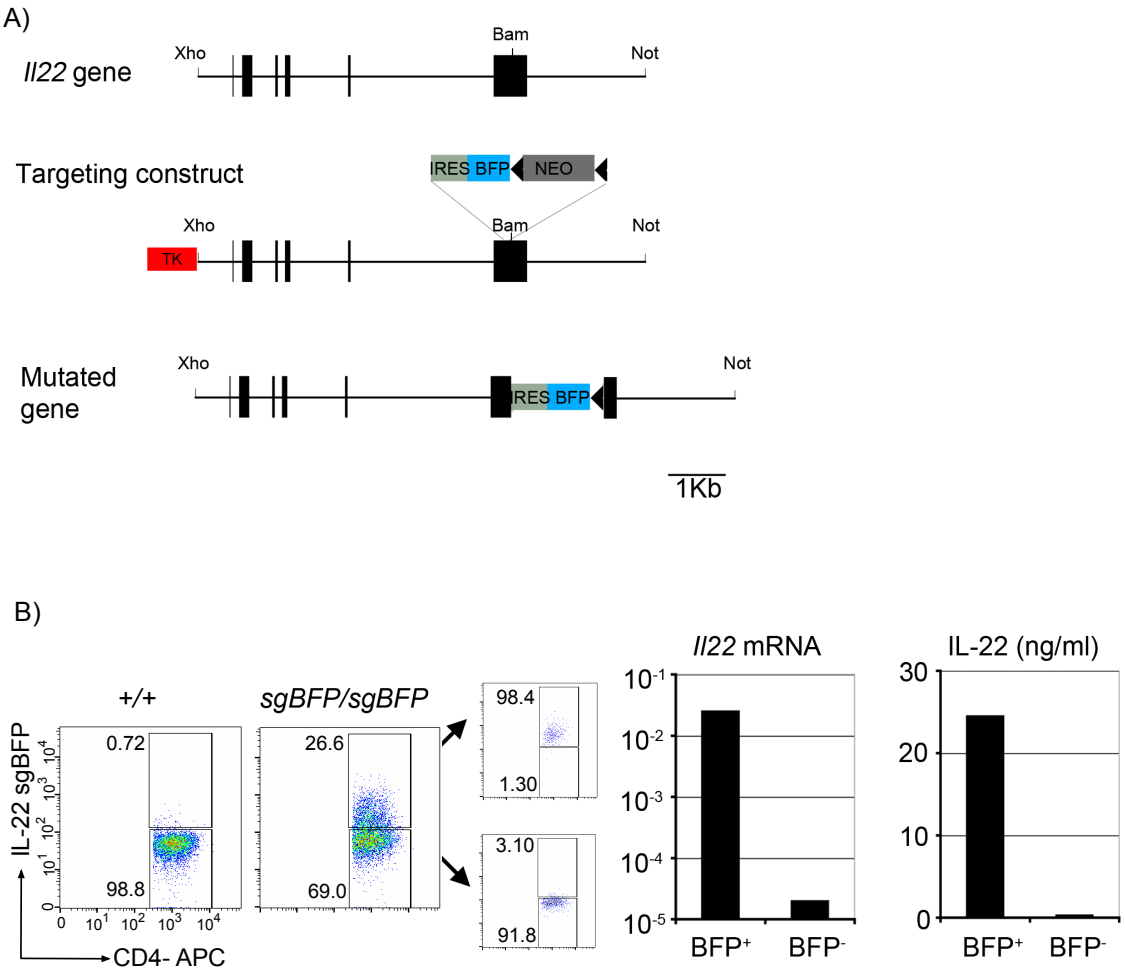
Perez et al.

# TGF-β signaling in Th17 cells promotes IL-22 production and colitis associated colon cancer

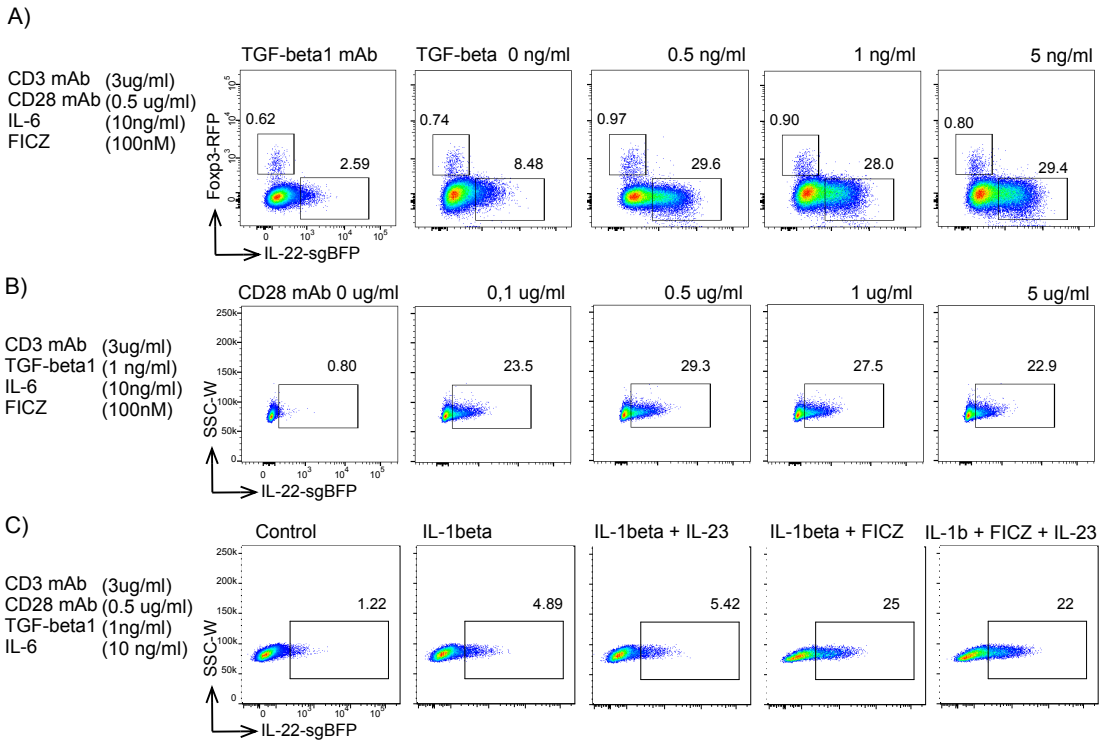
Supplementary information



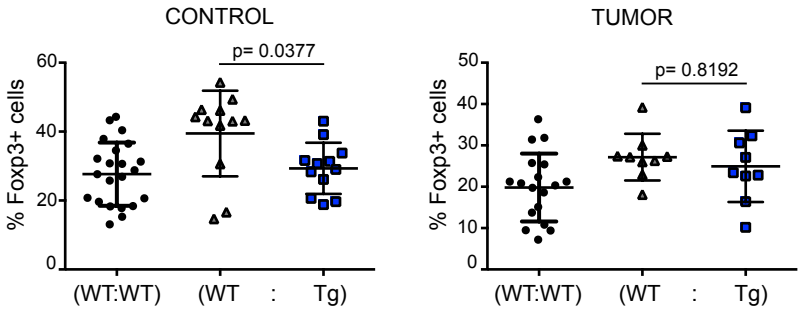

**Supplementary Figure 1: TGF-β1 promotes IL-17A production *in vitro*.**
Naïve T cells were isolated from spleen and lymph nodes of wild type (C57BL/6J) mice and cultured for four days in indicated conditions. IL-17A protein level was measured from culture supernatant (same as used in Figure 2a) by CBA, mean of technical duplicates is shown. Source data are provided as a Source data file.



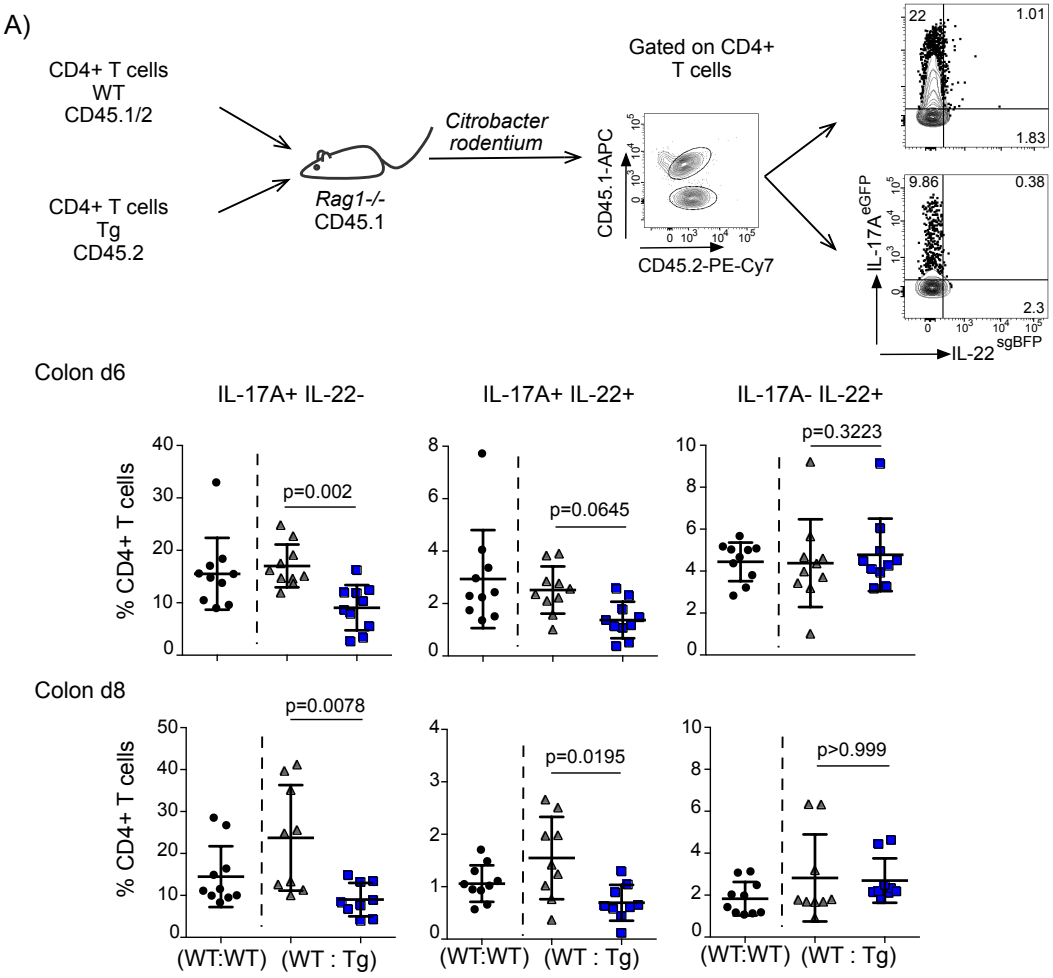

**Supplementary Figure 2: Generation and validation of IL-22$^{sgBFP}$ reporter mice.**
**A**) Targeting construct. **B**) Naïve T cells were isolated from wild type and IL-22$^{sgBFP}$ reporter mice and cultured in the presence of IL-6, IL-23, and TGF-β1 for four days. IL-22$^{sgBFP}$ positive and negative cells were sorted using FACS. *Il22* mRNA expression was measured using RT-PCR. IL-22 protein levels were measured in cell culture supernatants of IL-22$^{sgBFP}$ positive and negative CD4+ T cells upon restimulation for 48h using ELISA, mean of technical duplicates is shown. Results are representative of three independent experiments. Source data are provided as a Source data file.




**Supplementary Figure 3: Low dose of TGF-β1 and anti-CD28 is sufficient for IL-22 induction.**
Naïve T cells were isolated from spleen and lymph nodes of Foxp3$^{mRFP}$ x IL-17A$^{eGFP}$ x IL-22$^{sgBFP}$ reporter mice and cultured for four days in indicated conditions. **A**) Concentration of TGF-β1 was titrated for optimal IL-22 production *in vitro*. **B**) Concentration of CD28 mAb was titrated for optimal IL-22 production *in vitro*. **C**) Evaluation of the additive effect of IL-1β and IL-23 with FICZ in the production of IL-22 *in vitro*. Results are representative of two independent experiments.

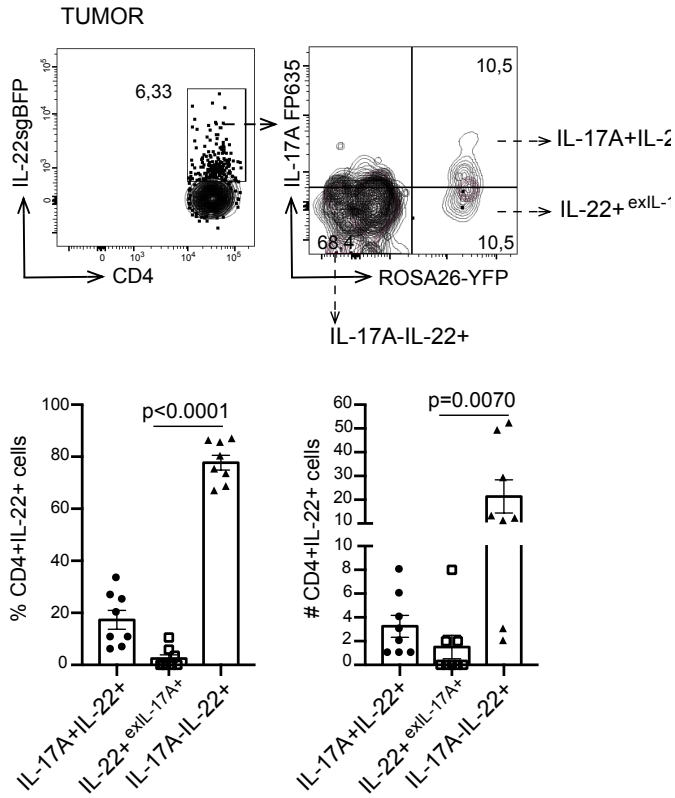



**Supplementary Figure 4: The role of TGF-β signaling for the emergence of Foxp3+ T cells in colitis associated colorectal cancer.**
CD4+ T cells from Foxp3$^{mRFP}$ x IL-17A$^{eGFP}$ x IL-22$^{sgBFP}$ (WT) or Foxp3$^{mRFP}$ x IL-17A$^{eGFP}$ x IL-22$^{sgBFP}$ x dnTGF-βR2 (Tg) mice were co-transferred into *Rag1-/-* prior to tumor induction. Frequency of Foxp3+ CD4+ T cells was analyzed by flow cytometry in both tumors and normal adjacent tissue (control). Results are cumulative from two independent experiments. Control: (WT:WT) n= 22; (WT:Tg) n= 12. Tumor: (WT:WT) n=18; (WT:Tg) =9. Lines indicate mean +/- sem. Two-sided Wilcoxon multiple comparisons test was performed ($P<0.05$) to assess the significance. Source data are provided as a Source data file.

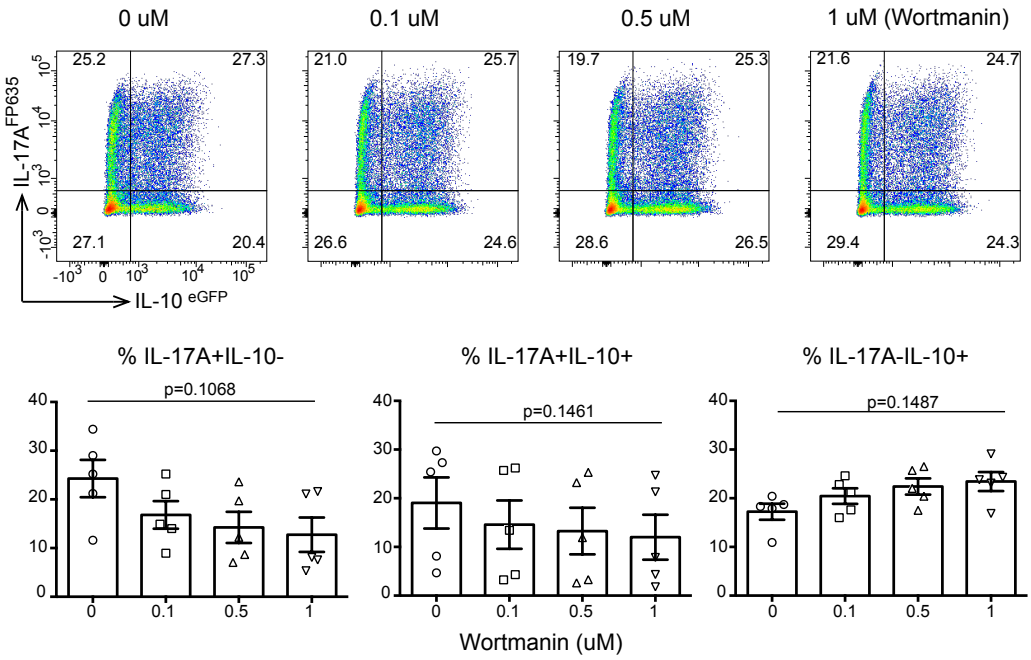



**Supplementary Figure 5: TGF-β signaling in T cells promotes the emergence of IL-17+IL-22+ CD4+ T cells in *Citrobacter rodentium* infection in a direct manner.**

**A**) CD4+ T cells from Foxp3$^{mRFP}$ x IL-17A$^{eGFP}$ x IL-22$^{sgBFP}$ or Foxp3$^{mRFP}$ x IL-17A$^{eGFP}$ x IL-22$^{sgBFP}$ x dnTGF-βR2 (Tg) mice were co-transferred into *Rag1-/-* prior infection. Production of IL-17 and IL-22 by T cells was analyzed by flow cytometry in the colon at day 6 and 8 post infection. Results are cumulative from two independent experiments. Colon day 6: (WT:WT) n= 10; (WT:Tg) n= 10. Colon day 8: (WT:WT) n= 10; (WT:Tg) n= 9 Lines indicate mean +/- sem. Two-sided Wilcoxon multiple comparisons test was performed (P<0.05) to assess the significance. Source data are provided as a Source data file.

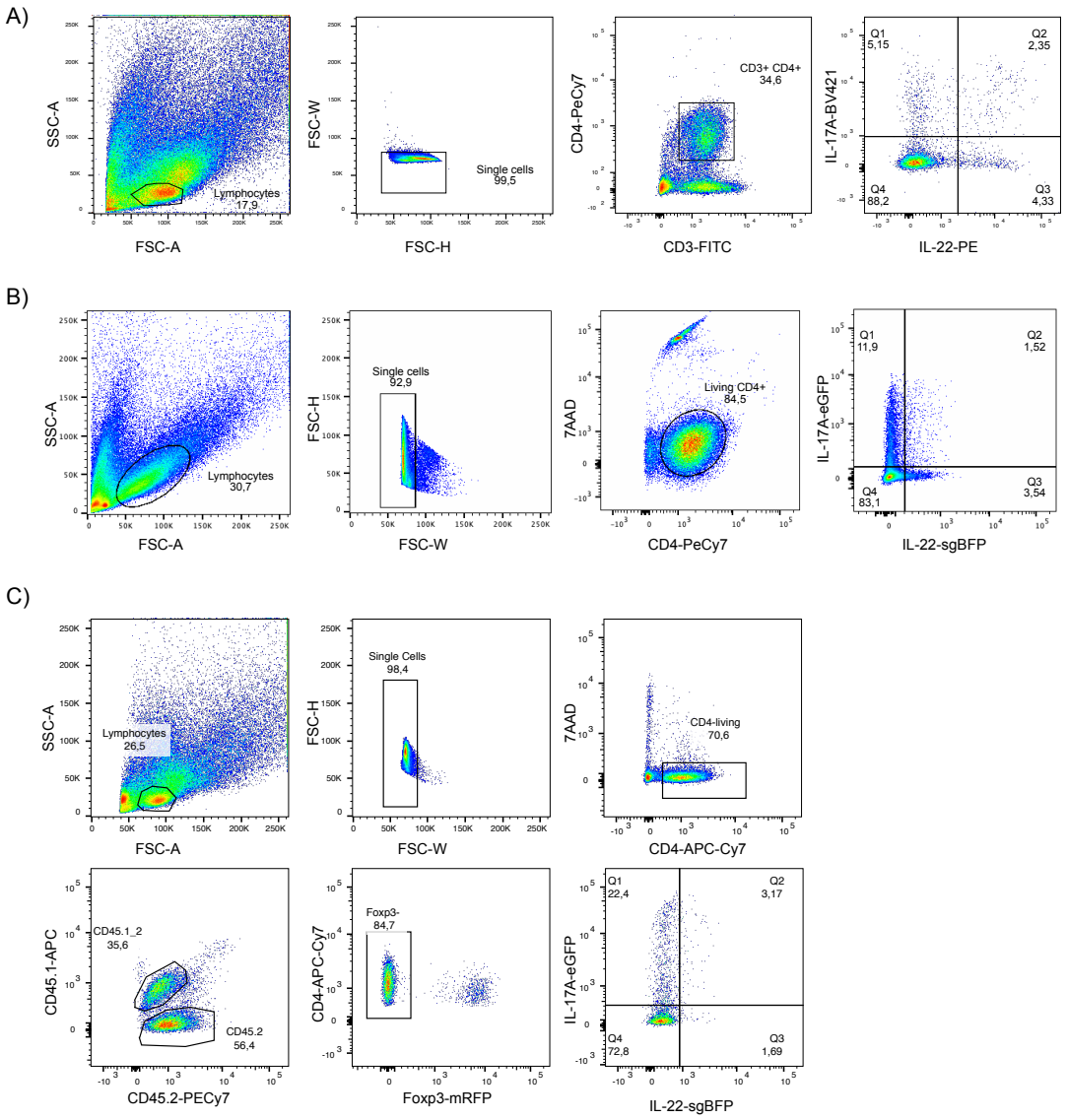



**Supplementary Figure 6: Fate of IL-22 producing T cells in tumors.**
Colitis associated colon cancer was induced in IL-17A$^{Cre}$ x Rosa26$^{YFP}$ x IL17A$^{FP635}$ x IL-22$^{sgBFP}$ mice. Lymphocyte isolation of tumors was performed and analyzed by flow cytometry. Frequency and cell number of indicated cell populations are shown. Each dot represents one mouse (n= 8). Bars represent mean, error bars show +/- sem. One-way ANOVA, Tukey's multiple comparisons test (P<0.05). Source data are provided as a Source data file.




**Supplementary Figure 7: PI3 Kinase activation is not essential for the emergence of IL-17+IL-10+ T cells.**

Naïve CD4+ T cells from Foxp3$^{mRFP}$ x IL-10$^{eGFP}$ x IL-17$^{FP635}$ x IL-22$^{sgBFP}$ reporter mice were differentiated under Th17 polarizing conditions and increasing amounts of PI3K inhibitor (Wortmanin). Frequency of indicated cell populations are shown, n=4, bars represent mean, error bars show +/- sem. Data are cumulative from four independent experiments. One-way ANOVA, Dunnett's multiple comparisons test. Source data are provided as a Source data file.




**Supplementary Figure 8: Representative gating strategies in flow cytometry analysis.**
**A**) Representative gating strategy of human flow cytometry analysis shown in Figure 1b. **B**) Representative gating strategy of flow cytometry analysis shown in Figure 2c, 5d and 6d. **C**) Representative gating strategy of flow cytometry analysis shown in Figure 3a and 4a.

**Table S1. Patients characteristics**

| | |
|---|---|
| sex (M/F) | 14/8 |
| age (years)* | 67 +/- 9.23 |

*mean +/- SD

**Supplementary Table 1. Colorectal cancer patients characteristics**